ATIONAL INSTITUTE OF SCIENCES OF INDIA
Symposium on Proteins in
Health

NATIONAL INSTITUTE OF SCIENCES OF INDIA

SYMPOSIUM
ON
PROTEINS IN HEALTH, DISEASE AND INDUSTRY

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ABSTRACT OF PAPERS

NATIONAL INSTITUTE OF SCIENCES OF INDIA
MATHURA ROAD, NEW DELHI

PREFATORY NOTE

The Council of the National Institute of Sciences of India, at their meetings held in October, 1953, at New Delhi and in May, 1954, at Calcutta, decided to organize a Symposium on "Protein in Health, Disease and Industry". A Steering Committee, consisting of Dr. K. V. Krishnan, Dr. B. Mukerji, Dr. M. Damodaran and Dr. U. P. Basu (Convener), was appointed to organize it. Accordingly, the Symposium was organized and **it will be held at the Institute of Science, Bombay, on Friday 6th August, from 2-45 to 5 p.m. and will continue on Saturday, 7th August, 1954, from 10 a.m. to 1 p.m. and from 2 to 5 p.m.**

The Committee took necessary steps to secure the co-operation of experts and persons and institutions interested in the subject. As can be seen from the Abstracts, the response has been extremely encouraging and the Organizers and the National Institute of Sciences of India wish to express their thanks to the contributors of papers and to the Institutions for the great interest they have taken in this Symposium.

Responses from the following institutions have been received and the abstracts of 44 papers are being published in this brochure.

A. National Laboratories.

1. National Chemical Laboratory, Poona ;
2. Central Food Technological Research Institute, Mysore ;
3. Central Leather Research Institute, Madras.

B. State Laboratories.

1. Indian Dairy Research Institute, Bangalore ;
2. Institute of Science, Bombay ;
3. Bengal Veterinary College, Calcutta ;
4. Health Directorate of West Bengal, Calcutta ;
5. Department of Fisheries, Orissa, Cuttack ;
6. Forest Research Institute, Dehra Dun ;
7. Haffkine Institute, Bombay.

C. Universities and Colleges.

1. University of Calcutta, Calcutta ;
2. University of Madras, Madras ;
3. St. Xavier's College, Calcutta ;
4. College of Engineering & Technology, Jadavpur ;
5. Central College, Bangalore.

D. Research Institutions.

1. Indian Cancer Research Centre, Bombay ;
2. Bengal Immunity Research Institute, Calcutta ;
3. Chittaranjan Cancer Hospital, Calcutta.

E. Medical Institutions.

1. All-India Institute of Hygiene, Calcutta ;
2. School of Tropical Medicine, Calcutta ;
3. R. G. Kar Medical College, Calcutta ;
4. Darbhanga Medical College, Bihar.

F. Industrial Organizations.

1. Industrial Chemical and Minerals Co., Ltd., Calcutta.

Note : It is requested that papers and summaries of remarks made during the discussions must be handed over to the Convener immediately after the meeting or as soon as possible thereafter.

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SECTION

A

INTRODUCTORY REMARKS

PROTEIN IN HEALTH, DISEASE AND INDUSTRY

The chemistry of proteins and its multifarious applications bewilder even any specialist in the line. The problems involve fundamental units in the protein molecule, methods of protein analysis, their structure, their physico-chemical properties, their applications to the problems of physiological interest and to different industries. The ubiquity of proteins in all manifestations of life puts them in the forefront of substances used in food, health, disease and industry.

Protein molecules are huge and complex. Their breakdown products may naturally give rise to various fragments upto amino acids. Just as the structure of a building is dependent upon the manner in which the bricks might be piled together, similarly interlinking of and with different amino acids may often give rise to different products. A glaring example will be found in the active principles of post pituitary lobe powder where the oxytocic characteristic of the octapeptide (consisting of cystine, tyrosine, isoleucine, glutamic acid, aspartic acid, proline, leucine and glycine) is completely changed to a pressor principle when isoleucine and leucine of the above peptide are, respectively, replaced by arginine and phenylalanine. Similarly, the formation of a protein molecule in complimentariness with a toxic or pathogenic antigen may help in protecting the body from its dreadful effects. Again, any abnormality in their mode of formation gives rise to serious disease like sickle cell anaemia (a hereditary haemolytic anaemia) where the haemoglobin molecule undergoes a structural change from normal haemoglobin. The cell-metabolism is virtually an enzymic function, and enzymes are proteins. A knowledge on the enzymic make-up of living cells would be helpful in any work for retarding the

growth of the micro-organisms. It may be noted that penicillin, the well-known antibiotic, is a dextro cysteine derivative. How the normal cell lives, grows, ages and dies and why one never ages and why it never dies until it has killed the host, are problems whose solution will save us from the dreadful thinking of cancer and other diseases. Similarly, proper fragmentations of a biological protein molecule may offer us nutrition in emergency, or, straightening the linking of amino acids in a complex protein from a waste, may offer us new fibres for the development of a textile industry.

In India we have already areas of 208 millions and 45 million acres of land for growing, respectively, food and non-food crops, about 300 millions of livestock, and over 14 million maunds of fishes (sea and fresh-water) besides other sources from which we may get protein and protein materials for our food and industry. Production in each category, however, needs expansion to ensure better health and to increase the economic prosperity of the country.

All these are fascinating and thought-provoking problems and some of these will be touched upon by the experts who have assembled here for a discussion on Protein in Health, Disease and Industry.

U. P. Basu,
Convener,
Steering Committee.

SECTION

B

FOOD PROTEINS, THEIR COMPOSITIONS AND NUTRITIVE VALUE

1. FISH PROTEINS IN HEALTH, DISEASE AND INDUSTRY *by* Kamala Sohonie and K. S. Ambe, *Institute of Science, Bombay.*

The muscles of fishes like sharks, skates, etc. which are extensively fished in Bombay water for the extraction of their liver oil, are cheaply disposed of as manure or poultry food. Experiments were carried out on such waste material and

proteins of high quality, free from fishy odour and of a light colour have been prepared.

They contain all the essential amino acids in considerable proportions and are very rich in the basic amino acids, deficiency of which is very common in our average diet. These proteins have been subjected to papyrographic micro-biological and enzymatic analysis to effect a fairly complete study from different angles. Considerable evidence has been collected to show that the fish proteins, quite comparable to casein, are of a high biological value.

Protein therapy is largely followed in illness as in health and it is hoped that the fish proteins prepared would be a cheap and effective substitute for the costly preparations like casein, fibrin and their hydrolysates, which we have to import today. These proteins also hold a very good promise in a number of industries such as, pharmaceuticals, confectionery, leather, dyes, textiles, resins, plastics, etc. apart from their use as supplementary food itself.

2. THE EFFICACY OF RICE PROTEINS FOR THE REGENERATION AND MAINTENANCE OF LIVER CYTOPLASM *by* M. V. Lakshminarayan Rao and M. R. Sahasrabudhe, *Central Food Technological Research Institute, Mysore.*

Rice glutelin is as efficient as casein for the regeneration of liver cytoplasm in adult male albino rats following a brief period of inanition. At a dietary level of 6%—the usual level at which rice proteins are present in the conventional poor rice diet—neither casein nor rice glutelin is able to maintain the level of liver cytoplasm when animals are transferred from standard to test diets. Supplementation of lysine to rice glutelin and of cystine to casein in the above diets helps to restore the liver cytoplasm levels to the normal. The liver cytoplasm levels in animals maintained for long periods on a poor rice diet are slightly lower than normal but significantly higher than those in animals receiving adequate diets containing rice proteins or casein at 6% level. This has been traced to the supplementary relationship between rice and tur dahl (*Cajanus indicus*) proteins, the latter contributing fair amounts of lysine to the dietary protein.

Chronic feeding of experimental animals on poor ri diets has been found not to impair the cytoplasm regenerati capacity of the liver.

3. RICE PROTEIN *by* M. N. RUDRA, *Darbhanga Medic College, Laheriasarai.*

Rice protein qualitatively is much superior to wheat pro tein. The methionine content of rice is about three time that of wheat (Rudra and Chowdhury, Nature, 166, 568). Thu the slight quantitative advantage of wheat protein is more tha offset by its poorer quality. The writer analysed some sample of rice with a high 10 per cent protein content. There can b no doubt that by suitable genetical modification rice havin a comparable quantitative protein content can be evolved. I is interesting to note that Hartman, Dryden & Cary (J. A Diet. A. 25, 929) reported Vitamin B_{12} activity in rice polish ings but no activity in wheat bran. Rice is poor in sodiun and calcium and has a greater acid balance. A rice diet there fore calls for a higher consumption of common salt and calciun normally but at the same time it is a great virtue in sufferer from hypertension.

4. STUDIES ON THE NUTRITIVE VALUE OF PLAN PROTEINS: PART I. PULSE PROTEINS—THEI UTILIZATION IN HEALTH AND DISEASE *by* G. C. ESH and J. M. SOM, *Bengal Immunity Research Institute, Calcutta.*

Pulses are the most important vegetable sources of ou protein foods grown in the country. Studies on their chemica composition, digestibility and biological values indicate tha they have limited nutritive value as compared with an ideal protein say egg protein. While their protein content varies from 20-30% and the digestibility from 85-90%, the biological value falls between 40-60% resulting the net utilization 37-58%. An analysis of essential amino acid contents also indicates tha all these pulse proteins are deficient in methionine and som are also low in tryptophane content.

Detailed investigations regarding the influence of these pulse proteins on the rate of growth and in protein regeneration were undertaken with rats as experimental animals. While considerable variation was observed in the growth promoting

value of different pulse proteins when weanling litter-mate rats were fed *ad-lib* amounts of diets containing equal sub-optimal amounts of protein (12%) provided by the respective pulses, the protein being the factor limiting growth in an otherwise adequate diet, methionine supplementation increased the protein efficiency ratio to a significant extent. It is interesting to note that when pulses are supplemented with threonine in addition to methionine and tryptophane much higher growth response has been observed than that obtained without threonine, indicating the influence played by threonine in the utilization of methionine and/or pulse proteins. When enzymatic hydrolysates were prepared from these pulse proteins and fed to protein depleted rats similar results in the protein regeneration were observed indicating the possibility of improving the nutritive value of pulse proteins for their better utilization.

5. BIOLOGICAL VALUE OF VEGETABLE PROTEINS IN RELATION TO THE MAINTENANCE OF LIVER CYTOPLASM *by* M. V. Lakshminarayan Rao and M. R. Sahasrabudhe, *Central Food Technological Research Institute, Mysore.*

A number of food proteins of plant origin have been evaluated with respect to their ability to maintain liver cytoplasm levels in adult male albino rats using the true protein content of the liver as the index of its cytoplasm content. Cereal proteins are as good as casein, while the pulse proteins are inferior to these. Supplementation with limiting essential amino acids, especially of methionine to the pulse proteins, considerably enhances the capacity of the proteins to maintain liver cytoplasm levels. Supplementary relations exist between cereal and pulse proteins.

The merits of the liver cytoplasm method for the nutritional assessment of proteins as compared with the classical methods will be discussed.

6. PROTEIN FROM GREEN LEAVES *by* P. R. Pal and B. C. Guha, *University College of Science and Technology, Calcutta.*

Fresh green leaves of 15 species of plants were analysed for their protein content. Of the leaves examined, the leaves

of drumstick, sesbania and ground-nut were found to co an unusually high concentration of protein, viz. 27·3%, 34 and 35·2% respectively on the dry basis.

In order to obtain the protein material economically f these sources, the following method was developed. Fi ground samples of leaves were extracted with 2% sodium bonate solution at room temperature (30°). The extracted tein was precipitated by adjusting the pH of the mediun the isoelectric point of the protein in question (this usu lies between 3·5 and 4) and heating to 80°. The precipi was centrifuged and dried. The yields varied between 40 70% of the total protein present. The crude protein was s ciently freed from colour and odour by extraction with 9 alcohol in order to be acceptable for human consumption.

The partially purified proteins from green leaves of dr stick, sesbania and ground-nut were hydrolysed with 6N and the amino acids present in the hydrolysates were dete by circular paper chromatography. The results which are gi in the following Table show that five essential amino a are present in drumstick leaves and six in sesbania ground-nut leaves.

TABLE

Amino acids in acid hydrolysate of leaf protein.

Amino acid	Drumstick	Sesbania	Ground
Lysine ...	... −	+	+
Serine ...	... +	+	+
Histidine ...	... +	+	+
Glycine ...	... +	+	+
Threonine ...	... +	+	+
Glutamic acid	... +	+	+
Alanine ...	... +	+	+
Proline ...	... +	+	+
Tyrosine ...	... +	+	+
Valine ...	... +	+	+
Phenylalanine	... +	+	+
Leucine ...	... +	+	+

The biological values of the proteins of the above three preparations are now being tested in animal experiments. The technological preparation of these protein materials appears to be feasible.

7. THE NUTRITIVE VALUE OF OILSEED CAKE PROTEINS

by S. Kuppuswamy and V. Subrahmanyan, *Central Food Technological Research Institute, Mysore.*

The chief importance of oilseed cakes as articles of human food lies in their protein value. A commercial expeller pressed sample of groundnut cake usually contains about 50% crude protein ($N \times 6{\cdot}25$) while similar samples of cottonseed cake (undehusked), sesame cake and cocoanut cake contain about 25, 35 and 20 per cent crude protein respectively. At a 10% level of protein intake, groundnut cake proteins have a B.V. of 56 and a digestibility coefficient of 90, while the corresponding values in the case of cottonseed cake, sesame cake and cocoanut cake proteins are 64 and 68, 55 and 87 and 70 and 85 respectively. As measured by the raw growth method, groundnut cake proteins have a protein efficiency ratio of 1·2 at 10% level of protein intake, cottonseed cake and sesame cake proteins, each a protein efficiency ratio of 1·0 and cocoanut cake, a protein efficiency ratio of 1·4.

Coming to the supplementary relationship between oilseed cake proteins and cereal proteins, it was found that in general, cocoanut cake proteins and groundnut cake proteins proved to be superior supplements to rice and wheat proteins as compared to the other two oilseed cake proteins.

A comprehensive programme of work on the effect of heat processing on the nutritive value of the proteins of oilseed cakes was also carried out. The nutritive value of the four oilseed cake proteins in the raw state was compared with that of these proteins after mild (steaming for 15 or 30 minutes), moderate (autoclaving at 15 lbs. pressure for 30 minutes) and drastic (autoclaving at 25 lbs. pressure for 30 minutes) heat processing, by the rat-growth method in the first instance. The experiments were later repeated by the nitrogen balance method using adult rats in order to find out whether the observed changes were due to corresponding changes in the digestibility of the proteins or the metabolism of the absorbed fraction or both.

The rat-growth experiments showed that there is definite improvement in the nutritive values of groundnut proteins on mild as well as moderate heat processing, though it is of a smaller magnitude as compared to the improvement in the nutritive value of soyabean proteins under identical conditions of heat processing. On drastic heat processing, however, there is a definite lowering in its nutritive value. On the other hand, on steaming cottonseed, sesame and cocoanut meals, or autoclaving them under mild conditions, the biological value of their proteins is impaired to varying extents, whereas the coefficient of digestibility remains essentially the same or is only slightly decreased. On drastic autoclaving, there is a fall both in the biological value as well as the digestibility. The significance of these findings is discussed in relation to modifying the procedure adopted in the oil-expulsion processes with a view to producing oilseed cakes of improved protein value.

8. NUTRITIVE VALUE OF FOOD YEAST PROTEINS AND THE SUPPLEMENTARY RELATIONS BETWEEN THE PROTEINS OF FOOD YEAST AND CEREALS

by Gowri Sur, Saranya Kumari Reddy, M. Swaminathan and V. Subrahmanyan, *Central Food Technological Research Institute, Mysore.*

Dried food yeast is a rich source of proteins and B. vitamins. The protein efficiency ratio of food yeast proteins was found to be 1·20 and 1·68 at dietary protein levels of 6% and 10% and the biological value and digestibility coefficient of the proteins at 6% level were 58·5 and 81·5 respectively.

The protein efficiency ratio of the yeast protein as determined by the rat growth method, was not affected by heat treatments like cooking, autoclaving or roasting. The digestibility of the protein as determined by *in vitro* methods showed that while cooked or autoclaved yeast is more digestible than raw yeast, roasted yeast is slightly less digestible.

The supplementary relations between yeast proteins and rice proteins were studied by Thomas-Mitchell nitrogen balance method, at 6% level of protein. These two proteins were found to supplement each other to a slight but significant extent.

The supplementary relations between yeast proteins and the proteins of some cereals were studied by the rat growth

method. Yeast proteins supplemented the proteins of wheat, jowar and ragi to a marked extent and rice proteins only to slight extent.

9. SOME NEW EDIBLE SOURCES OF PROTEIN *by* N. Subramanian, M. V. L. Rao and M. Srinivasan, *Central Food Technological Research Institute, Mysore.*

In our investigations on "Little Known Foods", we have found that the seeds of *Amaranthus paniculatus*, Linn, of bamboo and of *Sesbania grandiflora* Pers are good sources of edible proteins. The biological value of these proteins both by growth and by Nitrogen Balance method has been determined. These values for *Amaranthus paniculatus*, for bamboo and for *Sesbania grandiflora* protein are respectively 73·7±1·25, 74·7±1·44 and 35·6±1·88. Thus the seeds of *Amaranthus paniculatus* and bamboo are good sources of dietary protein, but not the seed of *Sesbania grandiflora*, in spite of its high protein content (68% of the decorticated seed). The low biological value of the *Sesbania grandiflora* seed is traceable to its very low content of methionine and lysine. Results obtained by supplementing this protein with casein as well as methionine will be discussed.

Amino acid make-up of these new proteins (as determined by Buffered Paper chromatographic technique) will be presented.

SECTION

C

PROTEIN PREPARATIONS IN FOOD AND THERAPY

1. PREPARATION OF A FORTIFIED COMPOSITE PROTEIN FOOD WITH MILK CASEIN AS THE BASE *by* V. Subrahmanyan, M. Swaminathan, M. V. Lakshminarayana Rao and S. Kuppuswamy, *Central Food Technological Research Institute, Mysore.*

Protein foods used in therapeutics may be broadly classified into protein hydrolysates and those containing whole proteins. Though protein hydrolysates are very useful in treating cases such as acute starvation, convalescence etc. where

the digestive capacity has been impaired, they suffer on the score of poor palatability and keeping quality. Preparations containing whole proteins, particularly sodium caseinate or other soluble forms of milk casein are extensively prescribed for expectant and nursing mothers and patients suffering from wasting diseases, protein deficiency diseases, general debility, peptic ulcer etc. Though products of this type are being imported in large quantities, no attempts have so far been made to prepare them in India primarily because of the difficulties encountered in concentrating solubilised casein.

The method for the preparation of a fortified protein food based on sodium caseinate has been standardised at the Central Food Technological Research Institute, Mysore and the process has recently been taken up by the industry. In brief outline, the process involves the conversion of casein into a soluble form in presence of minimal amounts of moisture, drying at a low temperature, grinding and sieving to obtain a fine particle size, incorporation of flavouring agents and fortification with minerals and vitamins. The proximate composition of the product so prepared is as follows: Protein, 82% ; Calcium 1·0% ; Phosphorus 0·6% ; Thiamine 10 mg.% ; riboflavin 10 mg.% ; Calcium pantothenate 10 mg.% ; pyridoxine 10 mg.% ; folic acid 4 mg.% and nicotinic acid 40 mg.%.

The product has a pleasant flavour and is highly palatable. It is easily dispersible in water or in milk and can be incorporated in a number of food preparations. Its therapeutic value has been demonstrated by extensive feeding trials with patients suffering from a variety of wasting diseases. Institution feeding experiments have shown that the protein food, when fed as a supplement to the diet of school children at the level of 1 ounce per day, produces a striking improvement in their growth and nutritional status within a period of eight weeks. The paper discusses these and other related results.

2. UTILIZATION OF WASTE FISH FLESH AS A CHEAP SOURCE OF EASILY ASSIMILABLE PROTEINS *by* G. B. Mohanty *and* A. B. Roy, *Department of Industries, Orissa, Cuttack.*

Hydrolysed protein, easily assimilable by human beings, soluble in water, rich in all principal amino acids, cheaper than

any available imported proteins, manufactured in powder form looking like milk powder from sources like shark fish which were being completely wasted before, now utilised in the most profitable way, with equipments like a spray drier and small laboratory accessories, already tested on several patients with satisfactory results, with a heavy demand in the market is being processed in our laboratory. Attempts are being made to manufacture it commercially.

3. LIVER PROTEIN IN FOOD AND THERAPY *by* S. K. Ganguly, *Bengal Immunity Research Institute, Calcutta.*

It has been observed that liver on enzymatic hydrolysis yields greater quantities of essential amino acids, vitamins of B group, minerals and haematopoeitic factors than from ordinary aqueous treatment of liver. An inter-relationship between the different amino acids and the haematopoeitic factors is evident from the better absorption and utilization of the protein of the liver as haemapoeitics.

The importance of such hydrolysis has been further discussed from nutritive as well as economic point of view.

4. NUTRITIVE VALUE OF THE PROTEINS OF A MILK SUBSTITUTE FROM GROUNDNUTS AND OTHER OIL BEARING SEEDS *by* M. N. Moorjani, D. S. Bhatia and V. Subrahmanyan, *Central Food Technological Research Institute, Mysore.*

A process has been worked out for the production of vegetable milk from groundnuts (*Arachis hypogea*). The industry has already taken up the process and the vegetable milk is now selling in certain parts of the country.

The nutritive value of the proteins of vegetable milk has been investigated in detail by a series of experiments. The protein of a milk substitute prepared exclusively from groundnuts has a lower biological value than cow's milk proteins but the nutritive value is enhanced by using a 75 :25 blend of groundnut and germinated soy-bean. The comparative efficiencies of the vegetable milk protein and casein for the regeneration of haemoglobin, red blood cells and for the maintenance of serum proteins have been studied. The vegetable milk

protein compares favourably with casein with respect to the formation of blood proteins. The vegetable milk proteins have been found to have a good supplementary effect to those of wheat.

Vegetable milk is most palatable in the form of curds (fermented product). The vegetable curd is almost indistinguishable from cow or buffalo curd. Changes in the nitrogenous constituents of groundnut milk during progressive lactic souring at 37°C have been studied over a period of 5 days. The total nitrogen content remained unchanged, while non-protein nitrogen and amonia-nitrogen increased and the protein nitrogen decreased as a result of souring.

It was observed that certain stored lots of groundnuts produced a milk which gave loosely set curds. Changes in the nitrogen constituents of groundnuts during storage were therefore studied and it was found that the groundnut proteins undergo a partial breakdown during storage. Denaturation of vegetable milk proteins as a result of the process of desiccation has also been studied.

5. IODINATION OF PROTEINS *by* V. Srinivasan, N. R. Moudgal and P. S. Sarma, *Biochemistry Laboratory, University of Madras, Madras.*

The discovery of thyroglobulin as the naturally occurring iodo-protein of thyroid gland about fifty years ago, can be said to have stimulated the study of iodo-proteins, resulting in the discovery of the various naturally occurring iodinated amino acids of considerable biological interest. Recently further impetus to the study of iodo-proteins was provided by the interesting work of Reineke and co-workers, who successfully prepared "artificial thyroglobulin" by iodinating casein and isolating thyroxine from it. The influence of these iodo-proteins on lactation both in cows and in lactating women has evoked considerable interest and in the present paper the results of various investigations carried out in the Biochemistry Laboratory, University of Madras on the iodination of waste proteins are presented.

Iodination of various waste proteins like groundnut cake, linseed meal, sesamum cake, cotton-seed meal, shark-ray collagen, cattle fibrin, haemoglobin, silk fibroin, etc. have been

carried out by the method of Reineke and Turner and their thyroxine content assayed both chemically and biologically. The thyroxine content was found to be characteristic of each protein and was not dependent on the tyrosine consumed or the total iodine content of the iodo-protein. The orientation of the di-iodotyrosine molecule in the protein appears to be the deciding factor for the thyroxine yield. Some of the di-iodotyrosine molecules may not be available for coupling due to unfavourable positions within the protein and the addition of extraneous di-iodotyrosine usually augmented the yield of thyroxine, probably by coupling with some of the di-iodotyrosine molecules.

Iodination of tyrosine and proteins in phosphate buffer led to many interesting results. The rate of iodine uptake, availability of tyrosine in the proteins for iodination and finally the thyroxine content of the iodinated product were all increased to a considerable extent. Incubation of di-iodotyrosine in phosphate buffer (pH 8·0) at 70°C was found to give a higher yield of thyroxine, than at the usual 60°C. Iodination of cattle fibrin in phosphate buffer (pH 8·0) resulted in iodo-protein containing 1·4% thyroxine, while that iodinated in bicarbonate medium contained only 0·5% thyroxine. By using phosphate buffer medium and Mn_3O_4 as catalyst, with an incubation temperature of 70°C, iodinated groundnut protein with a thyroxine content of 0·48% has been prepared and this can serve as a cheap source of iodo-protein for feeding milch cows.

A chemical method for the determination of thyroxine in iodo-proteins based on the colour reaction between diazobenzene sulphonic acid and thyroxine has also been worked out and the results obtained compared very favourably with bioassay values obtained on the same samples.

6. NUTRITIONAL APPRAISAL OF PROTEIN HYDROLYSATES FOR THERAPY *by* G. C. Esh, *Bengal Immunity Research Institute, Calcutta.*

Protein hydrolysates are nowadays available both for oral and parenteral administration for replenishing the protein depletion during many pathological conditions and physiological disorders. The nutritional efficacy of such preparations depends primarily on (*a*) protein source (*b*) nature of digestion

—acid, alkali or enzymic (*c*) processing factors mainly heat-treatment and adjuvants (*d*) degree of hydrolysis and others. While an estimation of the essential amino acids by chemical or microbiological methods gives only a rough idea on the nutritional efficacy of the product, it does not ensure that the protein equivalents contained in it will be biologically utilised suitably for the protein regeneration or replacement because of the fact that essentiality varies with the pattern of amino acids offered to the patients and that there exists some interrelationship between essential and non-essential amino acids. Moreover, some preparations contain amino acids in such a form that can be estimated by chemical or microbiological methods but are not always utilisable by higher animals including man.

The most practical and convenient method of assaying the nutritional efficiency lies with the rat test. There again various methods such as rat growth test, rat depletion and repletion test and intravenous rat depletion test can be taken up. Data have been presented to show that oral rat repletion test has some advantages over the growth test because it is done on the basis of weight recovered as well as plasma protein regeneration. Besides, intravenous rat depletion test is also being developed to confirm the suitability of the oral test as well as to judge the efficacy of parenteral preparations made with different degrees of hydrolysis. The whole problem is discussed.

7. EFFECT OF ADMINISTERING IODINATED CASEIN TO GROWING HEIFERS AND MILCH CATTLE UNDER INDIAN CONDITIONS *by* NOSHIR N. DASTUR, S. C. RAY and K. C. SEN, *Indian Dairy Research Institute, Bangalore.*

Studies have been carried out with young calves and adult lactating animals of indigenous and cross-breeds to see the effect of iodinated casein treatment under Indian conditions. Calves fed iodinated casein at the rate of 1·25 g. and 0·75 g. respectively per 100 lb. live-weight showed a higher growth rate than the control group. At the end of 20 weeks experimental period, the difference was found to be statistically significant. On the other hand, when the dose was raised to 2 g., there was a marked retardation in growth ; the gain in weight per head per day was only 0·4 lbs. as compared to 0·7 lb. registered

by the control animals not receiving thyro-protein. Metabolic studies showed that in the latter case, induced hyperthyroidism accelerated the catabolic processes, and the ingested nutrients were used up for purposes other than growth.

Administration of 2 g. of iodinated casein/100 lb. live-weight to milch cows showed increase in milk yield, which was however transitory. At the peak of response, the average increase was 13·1%. On the whole the response was higher in the cross-breds than in pure indigenous stock. The highest individual response was 39·4% in relation to the normal lactation curve. Fat %, total fat out-put and solids-not-fat % in milk also increased to the extent of 29·6, 22·9 and 5·6% respectively during the period of treatment.

8. HYPOPROTEINEMIA AND PROTEIN HYDROLYSATE *by* P. K. Guha, *Bengal Immunity Therapeutic Ward, R. G. Kar Medical College, Calcutta.*

In a survey of 690 cases admitted to the Bengal Immunity Therapeutic Ward of the R. G. Kar Medical College, Calcutta, it had been found that about 9 per cent suffered from hypoproteinemia. A historical account from the patients indicated that most of them had previously suffered from dysentery. Treatment with enzymatic protein hydrolysate (oral) 80 to 150 ounces and infusion of 100 to 900 c.c. 3 to 4 times a week showed improvement in most of the cases. Serum protein content raised from 3 to 5 gm. per cent to 5·7 to 6·4 gm. per cent. No adverse reactions nor any side effects were noticed.

9. FIBRIN HYDROLYSATE FOR PARENTERAL ADMINISTRATION *by* N. K. Dutta and K. C. Thadani, *Haffkine Institute, Bombay.*

Hydrolysates are prepared from various proteins for parenteral administration in patients suffering from hypoproteinemia and other related diseases.

We have prepared hydrolysate from Fibrin, one of the blood proteins. The amino acid composition of Fibrain hydrolysate has been estimated by microbiological and chemical methods and compared with that of Casein hydrolysate. The nutritive value of Fibrin hydrolysate has also been determined on adult rats.

Preliminary clinical trials have shown favourable results.

SECTION

D

BACTERIAL PROTEINS AND THEIR PROPHYLACTIC USE

1. SPECIFIC SOLUBLE PROTEINS OF PLAGUE ORGANISMS AND THEIR PROBABLE USE IN IMMUNISING MAN AGAINST PLAGUE INFECTION

by S. C. Seal, *All-India Institute of Hygiene and Public Health, Calcutta.*

As in the realm of crystals, variation in structure in the living organisms is dependent upon the difference in their chemical constitution. The search for an explanation of the remarkable phenomenon that recovery from an infectious disease is generally followed by immunity led to the discovery of antibodies and eventually of antigens stimulating their production, both of them having in common the property of specificity. The infective bacteria ordinarily contain multiple antigens in the form of proteins, polysaccharides, lipoids and phosphatides etc. singly or in combination. These may be obtained from the bacterial body or from its metabolites (e.g. toxin), but only one or a few of them are capable of inducing protective antibodies. For the production of an effective vaccine and to assess the results of immunisation it is necessary to separate the relevant from the irrelevant antigens. The search for the relevant immunising antigens has already been successful with organisms like pneumococci, streptococci, gonococci, Friedlanders bacillus, anthrax, typhoid bacillus, brucella, salmonella, dysentery bacilli and cholera vibrio etc. Recently, this work was extended to plague bacilli by the author in India followed by Baker et al in U.S.A. Several workers in the past had found this substance to be a protein but they did not succeed in obtaining a pure antigen perhaps due to the contamination of the substance with the non-specific proteins present in the media used. The author overcame this difficulty by introducing the modified casein hydrolysate broth, a protein-free liquid medium for growing the organism.

The virulent and avirulent plague and certain pseudo-tuberculosis bacilli were studied for specific antigen. Five fractions were isolated from the filtrates of the growth of these organisms in casein hydrolysate broth by precipitating at ⅓rd, ½, ½-⅓rd and 1-½ saturation of Na_2SO_4, the residue being taken as the last fraction. The first two fractions which formed 75% of the bulk were serologically active and the rest were inactive, the first fraction ($P^{\frac{1}{3}}$) forming about 90 per cent of the active part. Similar active protein fractions were also isolated from the water-soluble extracts of the virulent plague bacillus grown on casein hydrolysate agar, reacting with antiplague serum at a dilution of 1 : 1, 280,000. This fraction was absent in avirulent non-protective plague and pseudo-tuberculosis strains. Chemically, they resembled nucleo-protein in character but a difference was noted in the tryptophane content ; solubility was low and no optical rotation could be obtained but in the ultra-violet absorption carve a bend was noted in case of $P^{\frac{1}{3}}$, fraction obtained from the virulent plague strain. Serologically, the $P^{\frac{1}{3}}$ fraction of this strain, called Antigen A, was different from the $P^{\frac{1}{3}}$ fractions of non-protective plague and pseudo-tuberculosis strains which were serologically identical with Antigen B and the boiled virulent plague bacilli. Both 'A' and 'B' antigens were extractable from the broth filtrate but only 'A' could be obtained from the soluble extracts. An anti-serum produced against antigen A agglutinated only protective plague strains and not pseudo-tuberculosis and non-protective plague strains. It had also specifically fixed the complement, no cross-reaction being observed with anti-serum against other protein fractions or the whole organism of the avirulent non-protective plague or pseudo-tuberculosis strains, thus confirming the findings of other serological tests.

On further analysis of these protein fraction a poly-saccharide yeilding a osazone resembling that of *arabinose* with a melting point of 166°—168°C was isolated. It was absent in the avirulent non-protective plague and pseudo-tuberculosis organisms and in their protein fractions. Serologically, this polysaccharide fraction reacted only with the antisera against virulent and protective plague strains and against antigen A described above. This latter antigen (the one obtained from the broth filtrate) protected mice, against 10—20 times the m.l.d.,

even in doses less than the equivalent yield from the antiplague vaccine, while those obtained from the avirulent non-protective plague and pseudo-tuberculosis strains did not protect the mice against the same infective dose of virulent plague bacilli, thus proving that the $P\frac{1}{3}$ fraction or antigen A is the specific antigen which was responsible for the protection given by the Haffkine plague vaccine. The $P\frac{1}{3}$ fraction obtained from the water-soluble extracts also protected mice but required a little higher dose. Perhaps, it would now be worthwhile to try this antigen for regular human immunisation instead of the Haffkine plague vaccine which has limited keeping qualities.

SECTION

E

ANALYSIS OF PROTEINS AND STANDARDIZATION OF PROTEIN PREPARATIONS

1. INFLUENCE OF FORMIC ACID ON HYDROLYSIS OF TISSUE PROTEINS *by* (Miss) S. U. Gurnani, U. S. Kumta, and M. B. Sahasrabudhe, *Biology Division, Atomic Energy Commission, Indian Cancer Research Centre, Parel, Bombay*-12.

A quick and efficient method for the hydrolysis of tissue proteins has been developed. It involves the treatment of the tissue with 85 per cent Formic Acid followed by refluxing with 2N HCl for 2 hours. Using the micro-biological assay method for estimating the amino acid it has been shown that the hydrolysis is complete within 2 hours and that the recoveries of added amino acids are very good. Recoveries of Tryptophane added to tissue or to glucose tend to be lower although 100 per cent recoveries have been obtained with pure tryptophane alone, treated with Formic acid followed by 2N HCl for 2 hours. This method has routinely been applied to study the amino acid composition of proteins from Liver, Serum and Tumour tissues with good results.

2. HISTAMINE AND HISTAMINE-LIKE SUBSTANCES IN PROTEIN HYDROLYSATE AND THE NATURE OF THEIR INFLUENCES *by* A. N. Bose, *Bengal Immunity Research Institute, Calcutta.*

In the course of critical evaluation of solutions of enzymic Protein hydrolysate in this laboratory, definite contractions of the isolated virgin guineapig uterus were recorded, which in comparison with histamine showed that the substance might contain a fairly high amount of histamine-like substances (10 micrograms per c.c. on average). With tests on chloralosed cats, the solution gave a definite depressor response, but the quantity of histamine-like substances (3·6 micrograms per c.c.) present appeared lower than that indicated by the uterus method.

Tests with preparations of guineapig ileum bathed in original Tyrode solution showed a strong stimulating activity by the protein hydrolysate, but the histamine equivalence of the solution was very low (0·43 microgram per c.c.).

With ileum suspended in Tyrode solution with half-calcium and low magnesium, which lowered the sensitivity to non-histamine-like substances, it was found that most of the plain muscle stimulating property and depressor activity of a protein hydrolysate, suitably prepared, is probably associated with substances different from histamine. Transfusion of a large volume of Protein hydrolysate to a chloralosed cat after lowering the blood pressure significantly with carbachol, showed no tendency to vasomotor shock ; rather, it caused a steady rise in the tone of the heart and blood pressure.

The experiments suggest that hydrolysis of protein, particularly meat, is likely to liberate substances which stimulate plain muscles to a significant degree, but are largely different from histamine. Whether these are associated with adenosine and adenylic acid system in meat hydrolysates is being worked out.

3. THE QUANTITATIVE DETERMINATION OF AMINO ACIDS SEPARATED BY BUFFERED CIRCULAR PAPER CHROMATOGRAPHY *by* K. Krishnamurthy and M. Swaminathan, *Central Food Technological Research Institute, Mysore.*

The separation of 17 amino acids has been achieved by buffered circular paper chromatographic technique using diffe-

rent solvent systems. Aspartic acid, glutamic acid, serine, glycine, threonine and alanine were separated with the use of phenol saturated with buffer of pH 12, as solvent on filter circle buffered at the same pH. m-cresol saturated with buffer of pH 8·4 gave a clear separation of alanine, arginine, tyrosine, histidine, valine, methionine and phenylalanine. For the separation of cystine, arginine, alanine, proline and tyrosine, n-butanol-acetic acid-water (40-10-50) was used as the solvent. A mixture of phenol-n-butanol-acetic acid-water (20 : 20 : 8 : 40) was used as solvent on filter circle buffered with pH 2 buffer for the separation of cystine and lysine. Phenyl alanine, leucine and isoleucine were separated using a mixture of benzyl alcohol and tertiary amyl alcohol (1 : 1) saturated with water. The chromatograms were developed twice in each case (for about 8 hours each time). They were dried by means of a hot-air blower and uniformly sprayed with an acetone solution containing 0·5 per cent ninhydrin and 5 per cent acetic acid and heated at 80°C for 5 minutes to develop the colour produced with the amino acids. Standard chromatograms were obtained by running a mixture of known amino acids of suitable concentration on the same paper, side by side with the unknown amino acids. The coloured band due to each amino acid was cut, the colour was extracted with 5 c.c. of 75 per cent alcohol and estimated in a Klett-Summerson colorimeter using 560 mμ filter. The method was used for the determination of amino acids in casein and was found to yield results in agreement with those reported by other workers.

4. METHODS OF ANALYSIS OF PROTEINS AND AMINO ACIDS *by* V. S. Govindarajan, *National Chemical Laboratory, Poona.*

This paper reviews the application of the modern techniques of partition and ion-exchange chromatography and specific decarboxylases to the analysis of partially and completely hydrolysed proteins. The use of enzymes of differing specificity for the isolation of different fractions of proteins and their significance in studying protein structure are discussed. Some of the results obtained with the above techniques in the National Chemical Laboratory are briefly presented.

5. ENZYMATIC AND PAPER-CHROMATOGRAPHIC STUDIES ON SKIN AND HIDE PROTEINS *by* S. M. Bose, *Central Leather Research Institute, Madras.*

The important proteins of animal skins or hides are (*i*) the fibrous type viz., Collagen, reticulin, elastin, keratin, myosin and myogen and (*ii*) the non-fibrous type viz., albumin, globulin and glycoproteins. It is of great interest to study under scientifically controlled conditions the specific reactions of proteolytic enzymes on the skin and hide proteins. The reactions of the protease isolated from the latex of madar plants (*Calotropis gigantea*) have been studied on the important skin proteins under various conditions and the mechanism of the unhairing-cum-bating action on the skin has been demonstrated. By the use of the raw latex or the protease isolated from it, a simple process has been developed for simultaneously unhairing and bating skins and hides. It was, however, noted that the success of the process depended to a great extent on the careful observation of the exact time when the hair became loose. Any lengthening of the time beyond hair loosening involved several unwanted reactions with the skin proteins resulting finally in the decomposition and putrefaction of the proteins and liberation of malodorous gases. The factors responsible for the enzymatic digestion of the proteins and of the intermediary decomposition products have been investigated. The nature of the amino-acids which are preferentially liberated owing to the partial hydrolysis of the proteins has been studied. The work has been extended to prepare the total proteins as well as the individual proteins from skins and hides and to study their amino-acids composition by the recent technique of paper-chromatography. Among the different techniques followed, two-dimensional paper-chromatography has been found to be most suitable for the assay of detailed amino-acids composition of skin proteins.

6. STUDY ON THE COMPOSITION OF BACTERIAL NUCLEOPROTEINS *by* S. K. Datta, *Bengal Immunity Research Institute, Calcutta.*

Bacterial Nucleoprotein could not be isolated according to the method of Mirsky and Pollister. It was noted that Cetyl trimethyl ammonium bromide (CTAB) formed a complex with

nucleo-protein. Under mild conditions from the disintegrated cells the nucleoprotein was isolated, but it was found to be heterogeneous, by paper ionophoresis and ultracentrifugation. Carbazole reaction also detected the presence of some polysaccharides in this fraction.

However the nucleic acid isolated from this heterogeneous nucleoprotein was found to be fairly homogeneous. Purified nucleic acid was found to be a mixture of both desoxypentosenucleicacid (DNA) and pentosenucleicacid (PNA) whose relative proportions varied at different stages of the growth. The mixture was fractionated under very mild conditions into DNA and PNA. The purines, pyrimidines, sugar and phosphorous contents in both PNA and DNA were found to be almost unchanged during the growth period of the organism. Paper chromatography was used for these quantitative analyses. Hydrolysing agents were HCl, H_2SO_4, $HClO_4$ and CF_3COOH.

If the organism be made resistant to an antibiotic purine and pyrimidine contents of the PNA underwent significant changes.

Statistical analyses of all the analytical results have been recorded and discussed.

SECTION

F

FUNCTIONS OF PROTEINS IN THE BODY

1. THE ROLE OF HORMONES IN NITROGEN METABOLISM *by* P. B. Sen, *University College of Science, Calcutta.*

The tissue proteins owe their origin to the proteins in food. Nitrogen metabolism is governed by various internal factors, that are interdependent. These are components of the metabolic pool, the enzymes and the hormones. The enzymes and many hormones are protein in nature. The synthesis of both protein and non-protein hormones, and enzymes are dependent on the character of the metabolic pool and the complex enzyme systems that are present in the tissue, whose pattern is deter-

mined by the availability of the nitrogenous components and on the hormonal balance. An inter-relation between all these factors is thus apparent.

Several hormones have been observed to have significant effect on the nitrogen metabolism. Administration of growth hormone produces a utilisation of protein and diminished excretion of nitrogen in the urine. The cortical steroids increase the excretion of nitrogen in urine. Increased catabolic process in the tissue, break down of amino acids, decreased synthesis of body proteins and anti-insulin action have been suggested as some of the possible mechanisms. The influence of insulin on the protein metabolism is probably indirect ; the better utilisation of carbohydrate serving as a source of energy for the synthesis of protein. The thyroid hormones affect the oxidative processes in the tissue by regulating the enzyme pattern and the mechanism of oxygen transport. Sex hormones have also been found to influence both anabolic and catabolic phases of nitrogen metabolism. Some of the enzyme systems that are influenced by these hormones have been studied biochemically and histochemically.

2. THE MECHANISM OF SPECIFIC DYNAMIC ACTION OF PROTEIN, WITH SPECIAL REFERENCE TO THE EFFECTS OF VITAMINS, HORMONES AND ENZYMES

by D. P. Sadhu, *Bengal Veterinary College, Calcutta*-37.

Heat increment after feeding was known to Lavoisier and Laplace. This was studied systematically and quantitatively by Rubner who found out that the heat increment was greatest after a protein meal and called it specific dynamic action (SDA). If the basal metabolism of an animal at thermal neutrality is 100 Calories a day, then if it consume 100 Cal. in the form of protein, its heat production will increase to 131 Cal. for the day. The extra 31 Cal. is the SDA of the 100 Cal. of protein. In calculating the SDA, heat increment after feeding is taken as the excess over the fasting heat output, although some workers take maintenance plane as reference base.

Various theories have been put forward to explain the mechanism of SDA, but none was found to be satisfactory. The heat liberated by SDA can be utilized only for increasing

body temperature and is therefore useful in cold countries ; but in the tropical climates of India this heat is not only not useful but positively harmful by increasing the heat load of the body in the hot and humid summer days. This is a problem not only in man but also in animals with special reference to the highly productive animals such as high milch cows. This heat cannot be utilised as work and is analogous to ΔS in thermodynamics, a tax on the entropy, an unavoidable free energy loss associated with the nutritional energy conversions. This indicated that the mechanism must lie in some irreversible reactions in the body. On analysing the SDA of individual amino acids Lusk noted that dicarboxylic amino acids had a very low SDA, while Lundsgaard noted that all amino acids had high SDA.

It was shown by the author that the controversy between the two schools lay in the nutritional state of the animal. The experiments were conducted on albino rats in a modified Regnault-Reiset apparatus. Pyruvic and lactic acids lowered SDA of glutamic acid and tyrosine, but not of glycine, since pyruvate acts as an amino-acceptor of glutamic acid and tyrosine which are active transaminatically. Pyridoxine behaved similarly by its action as cotransaminase. Pyridoxine deficiency raised SDA of glutamic acid, but not of glycine. This work on transamination proved that SDA of amino acids is due largely to deamination in liver, which is counterbalanced by the simultaneous transamination in which amino group is accepted by an alpha keto acid, the latter being converted in its turn into amino acid. Transamination as a reversible process reduces the irreversible steps of deamination which cause energy loss as heat and thereby reduces SDA of amino acids.

Role of transamination in SDA is a contribution to a new concept of SDA and this explains all the unexplained and anomalous findings of the previous workers in the field.

3. SERUM AMYLASE ACTIVITY IN HYPOPROTEINEMIA *by* K. L. Mukherjee, *School of Tropical Medicine, Calcutta.*

The report is based on 28 pairs of estimations for serum amylase and serum protein concentration in patients suffering from nutritional oedema.

The amylase activity of the serum varied over a range of 33-261 Smith-Roe units in the patients and the serum albumin concentration showed a variation between 1·1 gm. and 4·5 gms.%, the higher values of the enzyme units corresponding to the higher albumin concentrations and vice versa. The correlation coefficient ($r=0·71$) between amylase activity (expressed in units) and serum albumin concentration was found to be statistically significant ($P=0·001$); however, linearity of regression could be secured only up to an albumin concentration of 2·5 gms.%. We were, therefore, led to the inference that a linear regression between albumin level and amylase activity of serum exists only within a limited range of the albumin concentration above which the enzyme activity is independent of the albumin concentration. In fact, the mean value of the amylase activity above 2·5 gms.% albumin in serum is practically identical with the corresponding value of normal controls (200 ± 39·9). Linearity of the regression of albumin concentration upon amylase activity was not obtained for the full range of data, even if one of the variables was plotted on a logarithmic scale.

4. PROTEIN UTILIZATION IN NUTRITIONAL AND HEPATIC DISORDERS *by* K. L. Mukherjee and G. Werner, *School of Tropical Medicine, Calcutta.*

Patients suffering from sufficiently far advanced nutritional oedema and patients with necrotic cirrhotic liver diseases are frequently in a very low positive or even negative nitrogen balance, even if kept on a high caloric, protein rich diet (standard diet of our cases: 3500 cal., 140 g. protein—mainly of animal origin—per day). A large part of their treatment consists in adopting measures which improve the protein utilisation.

The following observations were made on the clinical material of the Carmichael Hospital of Tropical Diseases within the last 1½ years:

(1) Cases of Nutritional oedema respond consistently with marked improvement of their nitrogen balance, if in addition to the standard diet glucose (100 c.c., 25 % intravenously) and insulin (10 units) are administered daily. A protein balance of

10-15 g. could on the average be achieved by that means in patients who before treatment were close to a balance of zero.

(2) Patients with necrotic as well as cirrhotic liver disorders showed marked improvement of their nitrogren balance, if Terramycin was administered (1·0 g. daily by mouth for prolonged periods, usually one month).

(3) Steroids with anabolic effect (Testosteron, 25 mg. twice weekly ; Methylandrostenediol 50 mg. daily) were not consistently found to improve the nitrogen balance of either nutritional or liver cases to a significant extent. Although there was usually a slight improvement of the nitrogen retention, statistical significance (in comparison with central periods) could in the long run not be secured.

5. THE REACTIVITY OF THIOL GROUPS IN PROTEINS *by* R. Lontie (Louvaia) and G. Beckers, *St. Xavier's College,* Calcutta.

A few proteins including Ovalbumine, Human, Bovine and Horse Serum albumine, Beta-lactoglobuline, Bovine Hemoglobine and Amandine have been tested by specific thiol groups reagents of different strength and type, both in their native state and after denaturation by urea.

So $Ag(NH_3)_2$ (amperometric), p-chloromercuribenzoate, ferricyanide, iodoacetic acid and iodoacetamide were allowed to react in standard conditions with a solution of each of those proteins.

Our data and those of the literature bring forth four types of phenomena requiring explanation :

I. Many a "masked" SH group reacts only after denaturation.

II. Often even spontaneous oxidation by air occurs after denaturation.

III. The reaction of the SH groups of the native protein differs with the sundry specific reagents.

IV. Individual differences sometimes appear for an identical protein treated by the same reagent.

I and II are explained in the light of the recent Pauling-Corey theories on protein structure. Besides, the hypothesis of intramolecular S-H-N bonds is put forward which will help to explain III. A set of independent experiments implying the reaction of cysteine and our specific reagents in presence of sundry substances support that hypothesis.

IV claims for greater precautions in the Standardization of methods. Explanations vary according to the different cases.

6. PROTEINS IN HEALTH AND DISEASE *by* M. N. Rudra, *Darbhanga Medical College, Laheriasarai.*

The young animal for its growing tissues and the adult for keeping him in nitrogen equilibrium require an adequate supply of proteins in the food. Additional protein is required for supplying the raw material for synthesis of non-protein nitrogenous bodies and sulphur and phosphorus compounds which are all necessary for our daily metabolic reactions.

In emergencies, like an operation, haemorrhage, pregnancy, lactation or disease, the protein requirement is greatly modified. Additional protein is required to make good the nitrogen loss and a further deposit against insurance. The proteins in these cases should be rich in the "essential aminoacids". This will help restoring nitrogen balance. Rudra (Indian M. J., 1938) has pointed out that in hard working normal healthy individuals, our knowledge about the indispensability of all the "essential aminoacids" may have to be modified. Legumes, the poor man's meat, containing the trypsin inhibitor factor may be improved biologically by easy, inexpensive processing methods.

The importance of methionine containing two biologically reactive 'thiol' and 'methyl' groups and of other amino acids in controlling biochemical reactions in the body both in health and disease is discussed.

7. PROTEIN AND RESISTANCE TO DISEASE *by* S. R. Sengupta, *School Health and Nutrition Division, Directorate of Health Services, West Bengal.*

Most authorities are agreed on the necessity for an adequate quantity of the protein constituent in the diet because of the

part which protein plays in the repair and renewal of exhausted tissues, and because tissue destruction and tissue wasting are clinically evident in chronic infective diseases. On clinical and epidemiological grounds, it has been indicated that both the quality and quantity of protein can influence resistance or susceptibility to certain diseases. However, it has been difficult, even in animal experiments, to distinguish protein from other nutritional factors that may be concerned in determining resistance or susceptibility to infection.

Some of the recent experiments have shown encouraging results with regard to salmonella and tuberculous infection and in all these, the important role played by protein in the diet has been stressed.

In my studies with tuberculosis and diet, the influence of diet on resistance to this infection was demonstrated in W-Swiss mice inoculated intravenously with a human strain of tubercle bacillus ; and it was found on analysis that the protein content of the diet on which the mice showed significantly higher resistance to the infection, was 19·2% compared to only 14·7% in the other diet studied, alhough on both the diets the mice grew and reproduced satisfactorily.

8. PROTEIN METABOLISM IN THE CELL OF THE EPITHELIAL TUMOUR *by* Subodh Mitra and Pradyot De, *Chittaranjan Cancer Hospital, Calcutta.*

The autonomous and destructive growth characteristics of malignant tumours, in comparision with normal tissues, may be caused by their abnormal metabolic pattern. Caspersson has shown that nucleoproteins are essential cellular constituents and participating in the formation of chromosome matrix and are closely related to the synthesis of the cytoplasmic proteins. He has also shown a close relationship between nucleic acid and protein metabolism in a great number of biological material, in the gene, in virus reproductions, during growth of bacteria and during growth and maturation of cells. Evidence has also been published indicating that disturbances in the nucleoprotein metabolism is the characteristic for malignant tumours.

High values (relative to normal tissues) for nucleoprotein content per unit volume of tumour tissues have been reported. But little is known about the functional connection between prosthetic nucleic acid groups and protein components.

The corrected ultraviolet extinctions (at 2650 Å and 2800 Å) per unit of dry weight shows moderate variations in the basal cell layers of non-neoplastic proliferating epithelium, but the extinctions are not significantly different from those in normal epithelium. In basal cell layers of pre-cancerous hyperplastic epithelium the extinctions at 2650 Å are somewhat higher than in normal epithelium. In slowly growing, highly differentiated epidermoid carcinoma the corrected extinctions at 2650 Å, per unit of dry weight are only slightly increased over the normal. In the three rapidly growing epidermoid carcinoma the corresponding extinction values are considerably higher in the infiltrating portions than in all other epidermoid tissues investigated.

SECTION

G

BIOSYNTHESIS AND SYNTHESIS OF AMINO ACIDS AND PEPTIDES

1. FORMATION OF ALPHA AMYLASE BY PANCREAS SLICE *by* Asoke Gopal Dutta, *Bengal Immunity Research Institute, Calcutta.*

While studying the action of antimalarial drugs on certain enzymes of the digestive system, it was noticed that addition of protein hydrolysate to surviving pancreas slice considerably increased the formation of alpha amylase.

In the present paper guinea pig pancreas slices were used for augmenting the formation of alpha-amylase from protein hydrolysate. The activity of the enzyme was measured by the method of Smyth and Roe, and formation of the enzyme was measured by determining the alpha-amylase content of the pancreas slices after incubation. The alpha-amylase activity in the medium after the incubation period gave an index of the rate of excretion.

It has been observed that antimalarials containing heterocyclic nitrogen ring like daraprim, supazine do not inhibit the formation of alpha-amylase whereas paludrine possesses some activity. Work with other nitrogenous antimalarials like quinine, chloroquin, primaquin is in progress.

2. POSSIBILITY OF PRODUCTION OF AMINO-ACIDS BY SYNTHETIC METHODS IN INDIA

by T. N. Ghosh and Saktipada Dutta, *Bengal Immunity Research Institute, Calcutta.*

In view of the growing demand of amino-acids as laboratory chemicals, in microbiological research and in nutritional therapy, the possibility of their production in India by synthetic methods should be explored. The synthetic acids, in general, are a racemic mixture of the optical isomers and the classical methods of preparing them include various routes, such as the Strecker synthesis, the malonic ester synthesis, condensation of aldehydes with reactive methylene group, etc. In recent years much development in the synthetic methods has been taking place, taking into consideration the availability and cost of raw materials, ease of synthesis, yields, etc. As for example, there are at least four processes for the preparation of dl-Methionine. They have been all tried in this laboratory and a critical survey shows that the following four-step process is the most feasible from all considerations: (1) condensation of acrolein with methyl mercaptan ; (2) reacting β-methylmercaptopropionaldehyde with sodium cyanide and ammonium carbonate ; (3) hydrolysis of the resulting hydantoin derivative with caustic soda to form dl-sodium methionate and (4) ultimately dl-Methionine. The major starting material, acrolein, may be obtained by the direct catalytic oxidation of propylene, available in volume from oil cracking. Methyl mercaptan, another starting material, would be a bye-product of gasoline refining.

Similarly, the problem of synthesis of dl-tryptophane may be attacked through several routes. These have been tried, modified and critically examined in this laboratory and the following process has emerged as the most suitable: (1) interaction of acrolein with acetamidomalonic ester ; (2) conversion

of the phenylhydrazone of the resulting product into the corresponding indole derivative and (3) hydrolysis of the indole derivative.

A survey of the possibility of manufacture of amino-acids like methionine, tryptophane, etc. by synthetic methods shows that their economic production would depend on the development of oil-refinery, cracking of oil, and availability on an economic basis of chemicals like sodium cyanide, acetic acid, aniline, etc. in this country.

3. BIOLOGICAL PRODUCTION OF FIBROUS PROTEINS

by M. SREENIVASAYA, *17th Cross, Malleswaram, Bangalore-3.*

Wool and silk are the two principal protein fibres which are produced through biological agencies. A close understanding of the intermediary metabolism involved in the formation of the protein would be helpful in controlling its biological production. There has been a considerable amount of work carried out mostly in Australia with regard to wool ; a daily dose of one gram of cystin intravenously administered to sheep, has been found to enhance the yield of wool by about 30 per cent.

The production of the silk by the silk worm involves the alimentation, assimilation and absorption of the proteins of the mulberry leaf and the subsequent synthesis of the constituent amino acids into silk protein by the gland of the silk worm.

The "essential" amino acids for the biological synthesis of the silk protein, are, glycine, alanine and serine. It has been found that the administration of these amino acids either along with the feed or by injection, enhances the production of the silk protein by about 20—25 per cent. The gland is an essential organ and constitutes the site of synthesis ; extirpation of the gland has been found to result in an accumulation of the amino acids in the haemolymph reaching toxic levels. Antibiotics, like chloromycetin, have been found to influence protein metabolism in general and in the case of the silk worm, the production of the silk protein.

SECTION

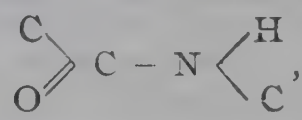
H

STRUCTURE AND MOLECULAR WEIGHTS OF PROTEINS

1. THE X-RAY CRYSTALLOGRAPHIC INVESTIGATION OF THE STRUCTURE OF PROTEINS AND THE PRESENT STATUS OF OUR KNOWLEDGE *by* A. B. Biswas, *National Chemical Laboratory of India, Poona*-8.

The elucidation of the sequence of amino acid residues in insulin by Sanger and his co-workers, and the new light thrown by Pauling and Corey on the nature of the spatial configuration of the polypeptide chain must rank amongst the greatest recent advances in protein chemistry.

Our knowledge of the molecular pattern of the protein is being rapidly advanced along two lines of approach by the X-ray diffraction methods. One relies on to determining the detailed structure of the simpler fragments of the protein molecule, whilst the other is making a direct attack on the entire protein molecule complex. The outstanding results of recent studies of this kind are reviewed.

In a programme of work along the former line, the detailed structure of a simple peptide, α-glycyl glycine, is determined and the results are described. The dimensions and configuration of the amide group,  , observed in this molecule and in its prototypes are critically discussed in relation to a possible stable configuration of the polypeptide chain. The results in general support the helical model, postulated by Pauling, for the polypeptide chain.

SECTION

I

PRESERVATION OF PROTEINS AND PROTEIN FOODS

1. PROCESSING OF MEAT FROM FISH OF CARP VARIETY *by* A. N. Bose, *College of Engineering and Technology, Jadavpur.*

Methods of dehydration and canning of crap-meat have been investigated. Moisture content of dehydrated crap-meat should be brought as near as possible but not below 2·5 per cent, for the best storage life of the product. Previous treatment of fish-tissue with steam at 5 p.s.i.g. for 15 minutes increased the drying rate over that with untreated fish-tissue. Best overall dehydration results were obtained by employing a temperature of 190°F until moisture content comes down to 52·2 per cent and then finishing at 160°F. The relative humidity should be maintained at 20 per cent for the incoming air. Using an air velocity of 500 ft./min and temperature and humidity conditions mentioned above, crap tissues of $\frac{1}{2}$ inch thickness can be dried to a moisture content of 2·5 per cent in 270 minutes. The chief drawback with this method of dehydration is the oxidation of fish oil which comes to the surface as the fish tissue shrinks during dehydration. Of the various anti-oxidants tried a mixture of 0·1 per cent α-tocopherol and 0·05 per cent l-ascorbic acid (as palmitate) in mineral oil was most effective in preventing oxidation.

In canning of crap meat, the skin and dark meat was found to be primarily responsible for objectionable flavour in canned product. Texture could be improved by treatment with 80-100° salometer brine. Flavour was greatly improved by pretreatment of fish to be canned, with 5 p.s.i.g. steam for 30 minutes.

SECTION

J

INDUSTRIAL APPLICATION OF PROTEINS

1. PROTEINS IN INDUSTRY *by* CHITTARANJAN BARA
Calcutta Industrial Chemicals & Minerals Co., Calcutta.

The employment of proteins in human economy could
traced back to the primeval ages. Apart from their nutrition
use, which man shares in common with the whole anim
creation, their economic exploitation must have started wi
the utilisation of animal hides and fibres as means of protecti
against the inclement extremes of nature. Such practices wou
naturally culminate into the development of methods for pr
cessing those native materials into stabler commodities leadi
ultimately to leather, wool and silk of our times. Strict
speaking, the processes of tanning or wool- and silk-scouri
fall under the category of Protein-technology in spite of th
apparent absurdity of such a generalisation. The developme
of gelatinous adhesives might have been an accidental find,
the outcome of the need of such joining materials by the grow
ing handicrafts of a still later age.

The importance of protein in various industries and th
implications involved in the manufacture have been presente

2. STUDIES IN PROTEIN-ALDEHYDE REACTION *I*
Y. NAYUDAMMA, *Central Leather Research Institut*
Madras.

The reaction between protein and formaldehyde has lon
been of both practical and theoretical interest to protein chemis
in many fields. As a result of the importance of this funda
mental reaction, many investigations have been undertaken o
this problem in the past. Much of the available data relativ
to this reaction cannot be compared with confidence due to th
differences in the materials and techniques used. This pap
presents results obtained in a comparative study of the differe
techniques used for the determination of bound and free forma
dehyde in the formaldehyde treated protein and the fixation
formaldehyde with specific reactive groups of collagen at variou
pH intervals and thereby elucidate the mechanism of tanning

3. PROTEINS IN THE TIMBER INDUSTRY *by* D. Narayanamurti, *Forest Research Institute, Dehra Dun.*

The development of modern adhesives has extended the use of wood and both technically and architecturally offers new possibilities. While synthetic resin adhesives with excellent properties have been developed in recent years the earliest adhesives used by man was animal glue, a protein. Protein adhesives still find wide application and in India 2000 tons of casein and a considerable quantity of animal glue are used every year. During the past 15 years extensive researches have been carried out at the Forest Research Institute, Dehra Dun on adhesives. In addition to the study of conventional adhesives like animal glue, blood albumin and casein this work has been directed to the isolation of proteins from agricultural and industrial wastes like gluten from starch factories, seed cakes and seeds of forest trees. Thus they deal with adhesives from albumins, globulins, prolamins and glutelins. Their rheological properties and their methods of application, strength, storage life, durability, etc. when used for joinery, laminating, or plywood, etc. have been studied. The results of these investigations are reported.

4. PREPARATION OF FAT-FREE PROTEIN FROM GROUNDNUT CAKE SUITABLE FOR PLYWOOD ADHESIVES *by* V. Subrahmanyan, S. Kuppuswamy, M. V. Lakshminarayana Rao and M. Swaminathan, *Central Food Technological Research Institute, Mysore.*

Proteins of both vegetable as well as animal origin are used in industry for diverse purposes such as the preparation of plastics, fibres, adhesives etc. India produces annually large quantities of oilseed cakes rich in proteins, but is grossly deficient in animal proteins with the possible exception of fish proteins. Even the oilseed cake proteins and fish proteins have not been utilized so far for industrial purposes to any extent. The only protein produced on a commercial scale in India is the milk protein, Casein—a costly material which is used in industry almost exclusively as a base for the preparation of plywood adhesives.

Proteins prepared by the usual methods from materials groundnut cake which are associated with fat invariably c tain a fairly high percentage of fat which renders it unsuita for a variety of uses in industry particularly the preparat of adhesives. Alkali extraction followed by acid precipita tends to concentrate the whole of the fat in the precipita material. The fat should be removed in the initial stages obtain a fat free protein. Solvent extraction of the isola protein or the oilseed cake brings about denaturation, there adversely affecting its solubility on which the industrial ap cation of the protein is primarily based. Investigations carr out at the Central Food Technological Research Institu Mysore, have shown that the use of solvents in the preparati of fat-free proteins can be avoided by skimming off the from alkaline extracts of oilseed cakes using a high spe separating centrifuge of the De Laval type. The process briefly as follows :—

The raw material is finely ground and extracted with 10- parts by weight of 0·1-0·2 per cent caustic soda or other alka like caustic potash, lime or ammonia of equivalent strengt The insoluble residue is separated using a basket centrifu and the clear extract is passed through a De Laval separato The rate of feeding is so adjusted that the volume of the oi fraction, flowing through the outlet at the centre of the bo is about 10-15 per cent the outflow of the oil-free fracti through the lower outlet. The latter fraction is collected a carefully acidified with a suitable mineral or organic acid t the iso-electric point is reached, when the protein separates ou It is filtered through a filter-press, dried in a current of hot a at a temperature not exceeding 45°C and ground. The prote prepared in this manner contains negligible quantities of fa

It was found that groundnut protein prepared according the above method from expeller pressed groundnut cake cou be used as a base of the preparation of plywood adhesive accor ing to a lime-caustic soda formula. The groundnut prote adhesive was slightly weaker in tensile strength than case adhesive, but sets less rapidly. However, groundnut prote could with a substantial reduction in cost, replace a portion the casein in the manufacture of plywood adhesive.

SECTION

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MISCELLANEOUS

1. STUDY OF TOXIC PROTEINS—VENOMS, *by* M. Sreenivasaya and M. D. Parthasarathy, *Department of Zoology, Central College, Bangalore.*

Animal venoms constitute an interesting class of proteins possessing toxicological and therapeutical properties. They are to be found distributed among widely separated phyla. Their occurrence and function vary in different animals.

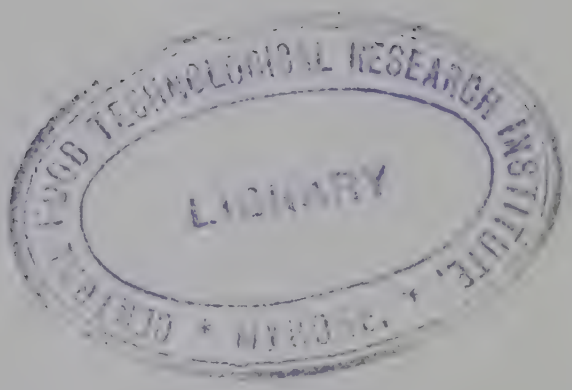
A biochemical study of the venoms which often occur in small quantities should necessarily be preceded by the development of special micro techniques, which we have been trying to develop. With a view to test the validity and applicability of these new techniques, we have undertaken a study of the venom of the scorpion. Most of the venoms have been found to flouresce strongly in the ultraviolet. They are precipitable by alcohol, acetone, glacial acetic acid, trichloracetic acid. Scorpion venom can sometimes be fractionated by paper partition chromatography by developing a streak of the venom on paper, with butanol-acetic acid or pyridine-water as the developing solvent. The venom in the scorpion has been found to exist in different degrees of aggregation as revealed by their separation on the chromatograms.

Acid hydrolysis of the whole venom in a sealed tube, reveals six different types of crystals which separate on concentration of the hydrolysate in a vacuum desiccator over silica gel and potassium hydroxide. These crystals are picked out individually by a micromanipulator, "spotted" on Whatman No. 1 paper and chromatograms developed with butanol-acetic-water mixtures, with a view to their identification.

Chromatograms of the acid hydrolysate of most of the venoms so far studied, reveal the presence of a ninhydrin-positive spot, bright scarlet in colour remaining "fast" for several weeks. Isolation and characterisation of this substance is under progress.

2. A SIMPLE AND INEXPENSIVE FRACTION-COLLECTOR *by* M. SREENIVASAYA, *17th Cross Road, Malleswaram, Bangalore*-3.

A simple and inexpensive fraction collector has been designed and constructed. A demonstration of this instrument will be given.



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